

PHARMACEUTICAL COMPOSITION WITH INCREASED BIOAVAILABILITY  
SUITABLE FOR THE ADMINISTRATION OF ORAL RETROVIRAL PROTEASE  
INHIBITORS. PROCESS FOR PREPARING A CONCENTRATE  
PHARMACEUTICAL COMPOSITION COMPRISING A RETROVIRAL PROTEASE  
5 INHIBITOR

The present invention describes a pharmaceutical composition with increased bioavailability, which is suitable for preparing microcapsules for the therapeutic administration of a protease inhibitor. It is also described  
10 a process for preparing a pharmaceutical composition with increased bioavailability, suitable to prepare concentrate pharmaceutical compositions comprising retroviral protease inhibitors.

The administration of drugs using soft gelatin capsules  
15 is a usual practice that is becoming more popular in the last years.

Typically soft gelatin capsules are comprised by a liquid containing the active pharmaceutical ingredient surrounded by an elastic gelatin pellicle. Due its elastic  
20 behavior that allows an easier ingestion, its acceptance among patients is higher than the conventional tablets or hard gelatin capsules.

Another important property derived from this kind of formulation is to allow the administration of a medicine  
25 wherein the pharmaceutical active ingredient is completely dissolved in a solution. Once ingested, the capsule breaks inside the gastrointestinal tract liberating the medicine in an homogeneous way, and as a liquid, it is not necessary first to be dissolved by the body in order to be absorbed  
30 later.

It is known that several active pharmaceutical ingredients show different absorption behavior inside the

biological environment, many times being dependent from their actual crystalline form.

Polymorphism is a physical-chemical characteristic that is very important in bioabsorption studies, since different crystalline forms of an active pharmaceutical ingredient (active ingredient) usually proportionate solubility differences, and so, different absorption rates by the organism. These absorption differences usually proportionate different plasmatic levels in the blood, conferring an erratic pattern in the minimum circulating level of the drug in the organism. As a consequence of this erratic pattern, those individuals treated are susceptible to subdosages or overdosages of these drugs.

Even subdosages as overdosages are negative therapeutic aspects, as they may lead to a complication in the clinical picture of the patient. In the case of a subdosage, the patient do not receive the minimum necessary dose of the drug for the stabilization or the cure of its disease, while in the case of an overdosage the patient absorbs a higher amount of the drug than the necessary, which can lead to a severe intoxication picture.

There are drugs that present an excellent effect "in vitro" but due their low solubility they are not adequately absorbed "in vivo" being considered as therapeutically ineffective.

Soft gelatin capsules allow the administration of several active pharmaceutical ingredients having the above-described characteristics as a pre-concentrate microemulsion, or even in the form of a solution, pharmaceutical presentations that make their absorption easier in the biological environment. Issues like low solubility and different crystalline aspects may be easily

controlled by using soft gelatin capsules allowing an adequate, reliable and predictable performance of the drug.

Besides the fact of the active ingredient is administered as a liquid medium, the contained excipients  
5 may help in other absorption characteristics, for example, promoting a medium wherein the drug can be absorbed easier because a pH control or because the presence of substances that can assist or promote the transport through specific  
10 membranes or cells of a specific part of the organism. These substances, called absorption promoters, can be introduced easily in these pre-concentrate microemulsions making the drug absorption easier by the organism and making the drug more effective.

Especially in the treatment of infections caused by  
15 virus or bacteria, a complicating factor is the development of resistant roots a reasonable occurrence when the active drug does not reach the infected target issue in a minimum amount necessary to the eradication or complete inactivation of the infectious agent. Resistance is without any doubt one  
20 of the biggest problems that may overcome during the treatment of diseases caused by infectious organisms and the efforts to avoid it are frequent.

There are several issues that a drug must comply in order to be considered adequate as a therapeutic agent.  
25 Among them, a drug must present therapeutic efficacy and for that it has to possess adequate characteristics of bioabsorption and bioavailability. Besides that, the drug must present an administration route less invasive as possible in order to avoid the patient to be submitted to  
30 painful and uncomfortable procedures during its administration. Another extremely important factor is that it has to stimulate or to motivate the patient adherence or adhesion to the therapy.

Adherence or adhesion to a treatment may be defined as the act, action or quality of being consistent with the administration of the prescribed medicaments. This issue is so more important as longer the time of duration of the therapy prescribed to a patient.

Nowadays, AIDS (Acquired Immunodeficiency Syndrome) treatment uses medicines that must be administered every day, many of them in high dosages and, which treatment can not be interrupted even a day for the rest of the infected individuals life. In the case of AIDS, antiretroviral therapy success depends mainly on the adherence of the patient to the therapy itself, this therapy consisting in the ingestion of significant amounts of medicines several times a day.

Accordingly with the final report named "Avaliação da Aderência ao Tratamento por Anti-retrovirais em Usuários de Ambulatórios do Sistema Publico de Assistência a AIDS no Estado de São Paulo" (a report evaluating the adherence of patients to antiretrovital therapy in the State of São Paulo - Brazil), non-adherence to new AIDS medicines (antiretrovirals mainly and protease inhibitors in particular) is considered one of the most threatening dangers to the effectiveness of the treatment, in an individual level, and for the dissemination of virus-resistance, in a collective level. That is because new AIDS administration therapies seems to request from the "adherent" a complex integration between knowledge, ability and acceptance, besides other important issues related to the environment and to the health care. The report discloses the matter of non-adherence being a universal phenomenon in a certain rate, occuring either in rich and poor countries, even in cases of life threatening diseases.

Adherence to antiretrovirals is a motive of apprehension among health professionals, once researches about it demonstrate that it is very low even in rich countries, where it reaches only 70% of the patients under treatment (Walsh J., Dalton M., Gill J., Wilkinson D. Burgess A.P., Gazzard B.G. - Adherence to protease inhibitor based highly effective anti-retroviral therapy (HAART) in 12<sup>th</sup> World Aids Conference, Geneva 1998. Abstracts; Hecht F.M., Colfax G., Swanson M., Chesney M.A. - Adherence and effectiveness of protease inhibitors in clinical practice in 5<sup>th</sup> Conf. Retrovir. Oppor. Infect., San Francisco, 1998 Abstracts, e Eldred L. - Adherence in the era of protease inhibitors - John Hopkins AIDS Service). This percentage is considered very low for a fatal disease, especially when considering that those reports had exclusively disclosed the adherence from patients to the amount of medicines recommended.

In particular, one of the components from the anti-aids cocktail corresponds to a special class of drugs, which acts by inhibiting the retroviral protease. Protease inhibitors are substances with high molecular weight, normally lipophilic, slightly soluble in water and usually present low absorption and low bioavailability "in vivo". Due those characteristics, elevated and frequent dosages of these substances are necessary to maintain an ideal therapeutic circulating level of the drug in the organism.

N-tert-butyldecahydro-2-[2(R)hydroxy - 4-phenyl-3-(S)-[[N- (2- quinolylylcarbonyl) -L- asparaginylyl]amine]butyl]-(4aS,8aS)-isoquinolone-3(S)-carboxamide, also known by its generic name saquinavir, was originally developed for the treatment of AIDS as a pharmaceutical composition using its mesylate salt. Some years after its launch, it was observed that patients treated with this medicine had presented

higher incidence of virus resistance development when compared to patients treated by other protease inhibitors. This feature was attributed to the poor and irregular absorption of this drug by the organism when using the pharmaceutical composition originally developed. Among  
5 several critical features considered about the composition, the presence of saquinavir in a solid form was considered to be the determinant cause for its low therapeutic efficacy.

The answer found for improving the biological activity  
10 of saquinavir was the development of a new composition, where it could be administered in a soluble way. Today saquinavir is marketed as its new composition known by the name of FORTOVASE™.

In accordance to FORTOVASE™'s monograph from the  
15 European Medicines Agency (EMA) it is necessary to use saquinavir as an amorphous powder for the preparation of this composition, because of the low solubility of saquinavir in the excipients employed. This requires the use of a controlled crystallization technique in order to avoid  
20 the emergence of well-defined crystals when considering its industrial preparation. Besides this important technical disadvantage, that demands the establishment of selective crystallization parameters to obtain this amorphous form, another much more critical problem resides on the physical  
25 behavior of this composition. This composition that is marketed within soft gelatin capsules, most of the times does not remain as an ideal liquid, changing itself into a thick gel. The emergence of this thick gel is aleatory and unpredictable and capsules affected by this problem present  
30 significant differences of "in vitro" dissolution when compared with normal capsules.

The physical instability of the current saquinavir composition interferes in the rate of availability or drug

liberation rate into the organism, as the capsules with this gelling problem will dissolve in different rates from the normal capsules, implicating in unpredictable differences of saquinavir bioavailability to someone in treatment. This  
5 problem is unacceptable considering the treatment of a viral infection where the erratic suppression of the virus can lead to the development of virus-resistance.

In the literature it is possible to find several references of pharmaceutical compositions for administering  
10 protease inhibitors. Among them there is the application that references FORTOVASE™, WO 96/39142, which describes the administration of some protease inhibitors in a composition comprising a triglyceride, which is a mixture of mono and diglycerides of fatty acids (i.e. capric and  
15 caprilic). Studies about the performance of this composition were done using as a protease inhibitor saquinavir as its base form only, and the composition was evaluated by comparing it with the saquinavir mesylate composition vehicled in hard gelatin capsules. As a result, soft gelatin  
20 capsules with saquinavir as its base form formulated in a concentrate microemulsion in the selected triglycerides showed a better bioavailability when compared to the hard gelatin capsule formulation with saquinavir as its mesylate salt, besides the better solubility of this salt form. The  
25 commercial realization of this invention is a soft gelatin capsule filled with about one gram of the concentrate microemulsion surrounded by a pellicle of soft gelatin. Due the great amount of the excipients necessary to dissolve the quantity of saquinavir per capsule (200mg of saquinavir  
30 base), the capsule is very huge difficulting the swallowing of it by patients, in special children and debilitated patients. However, this inconvenience cannot be compared to the very serious instability problem of this composition described before, which can lead to an unpredictable

absorption and bioavailability of saquinavir that can contribute extensively in the development of virus-resistance.

Another reference is application US08/754,390, which  
5 describes a pharmaceutical composition comprising ritonavir, a long chain fatty acid and an acceptable surfactant. Despite mentioning the possible use of saquinavir, this reference does not have any example about the preparation of a composition comprising saquinavir, only examples  
10 describing the composition using ritonavir as the active pharmaceutical ingredient. Experimentally, the preparation of a saquinavir concentrate using that predicted excipients is not feasible in such high concentrations because it is impossible to achieve its complete dissolution and there is  
15 a considerable decomposition of their constituents derived from a long period of intense heating that the composition has to be submitted.

Patent US 6,299,906 describes a process for the preparation of submicronized particles of some biologically  
20 active compounds that are normally slightly soluble, among them saquinavir, in order to increase considerably their solubility. The process consists in the dissolution of the biologically active substance under elevated pressures within a compressed gas, liquid or supercritical fluid containing a  
25 surface modifier, and expand the compressed solution as fast as possible in order to crystallize the dissolved compound. This procedure demands the acquisition of very expensive equipments and do not enhance considerably the solubility of saquinavir. This patent does not describe any pharmaceutical  
30 composition using this submicronized saquinavir either.

Application WO 98/57648 describes methods to enhance the bioavailability of crystalline polymorphs from several compounds, among them saquinavir. The technique comprises



the manufacture of nanoparticles with an average size inferior than 400nm. The authors claims that because of the reduced size of the particles, pharmaceutical compositions manufacture with the biologically active agent becomes  
5 easier and the composition more bioavailable, mainly when presented as suspensions. As in the case of the above reference, this kind of process requires the inclusion of an extra step in the manufacture, besides the need of special equipments. There is not any suggestion for preparing a  
10 pharmaceutical composition, as well as any test or result that demonstrates its effectiveness on enhancing saquinavir bioavailability.

There are also several references describing the preparation of pre-concentrate microemulsions suitable for  
15 the encapsulation in soft gelatin capsules, but none of those references describes compositions containing saquinavir.

One of the objectives of this invention is a pharmaceutical composition comprising a stable pre-  
20 concentrate microemulsion wherein saquinavir is soluble, suitable for the encapsulation in soft gelatin capsules or hard gelatin capsules for oral administration in the treatment of AIDS.

It is also another objective of the present invention  
25 to demonstrate that the pharmaceutical composition of the present invention presents a bioavailability profile much higher than the available marketed composition, being possible to use an adequate therapeutic dose considerably inferior than the therapeutic dose necessary until now.

30 Another objective of the present invention is the process for preparing a pharmaceutical composition comprising a concentrate microemulsion of saquinavir, a process that allows to obtain a concentrate microemulsion

with high concentration of the active pharmaceutical ingredient, allowing the preparation of capsules or microcapsules comprising the adequate therapeutically amount of saquinavir.

5        Nowadays saquinavir in its marketed pharmaceutical composition as soft gelatin capsules must be administrated in a daily dosage of 3,600mg, distributed in three intakes of 1,200mg each. Soft gelatin capsules available in the market comprise saquinavir in an amount of 200mg/capsule,  
10        being higher concentrations not prepared because it is impossible to dissolve or to prepare a stable pharmaceutical emulsion with the excipients proposed. So, the therapy consists in the ingestion of six capsules three times a day, making up a total amount of eighteen capsules taken a day.  
15        By this regularity, a treated patient must have taken an amount of 540 capsules at the end of a month, an extremely elevated quantity mainly when considering that besides this medicine other components of the anti-AIDS cocktail are also need to be taken.

20        Besides using saquinavir necessarily in its amorphous form, this composition presents other several ingredients that are necessary not only for their stabilization, but also to promote its dissolution within the composition. Due the low solubility of saquinavir within the selected  
25        excipients, the required amount necessary for its dissolution is extremely high in the adequate therapeutic dosage. By consequence, each saquinavir capsule commercially available contains the concentrate with the active ingredient in a final quantity of about 1,000mg of the  
30        composition comprising an amount of 200mg of saquinavir. This amount of the composition is enclosed within oblong gelatin capsules N° 20, that present a voluminous size, making their ingestion difficult by patients submitted to

therapy, mainly when considering patients with several infections of the digestive tract (normally present in acute cases of the disease) and to the children that are not able to swallow them because of its huge size.

5       The pharmaceutical composition of the present invention comprises the following ingredients:

- (i)       N-tert-butyldecahydro-2-[2(R)hydroxy - 4-phenyl-3-(S)-[[N-(2-quinolylylcarbonyl)-L- asparaginylyl]amine] butyl]-(4aS,8aS)-isoquinolone-3(S)-carboxamide  
10       (saquinavir) as its free base, or its pharmaceutical acceptable salts, as the active ingredient;
- (ii)      A fatty acid with a chain of C<sub>12-18</sub>;
- (iii)     At least an alcohol of C<sub>2-4</sub>;
- 15       (iv)   At least a non-ionic surfactant;
- (v)       At least a pharmaceutical acceptable antioxidant.

The pharmaceutical composition of the present invention consists in a concentrate microemulsion containing as the active ingredient N-tert-butyldecahydro-2-[2(R)hydroxy - 4-phenyl-3-(S)-[[N-(2-quinolylylcarbonyl) -L- asparaginylyl]amine] butyl]-(4aS,8aS)-isoquinolone-3(S)-carboxamide, which is  
20       known by the generic name of saquinavir, in its base form or as an acceptable pharmaceutical salt. Saquinavir, or a pharmaceutical acceptable salt of it, is used in a  
25       concentration preferably ranging from 10% to 80% in weight of the final composition. More preferably yet, saquinavir is used in a concentration ranging from 15% to 70% in weight of the final composition.

30       In this concentrate microemulsion composition the long chain fatty acid of C<sub>12-18</sub> presents the property to provide a hydrophobic medium adequate to avoid a possible precipitation of the active ingredient from the composition.

The preferred fatty acid with a chain of C<sub>12-18</sub> is the oleic acid, which can be used in a concentration ranging from 20% to 80% in weight of the final composition, more preferably in a concentration ranging from 20% to 70% in weight of the  
5 final composition.

Saquinavir solubility and/or of its pharmaceutical acceptable salts is strongly favored by the additional presence of an alcohol of C<sub>2-4</sub>, especially ethanol and/or propylene glycol. The combination of the fatty acid with one  
10 of this alcohols allows a medium wherein the completely dissolved active ingredient presents a great stability, specially if the composition does not have the presence of microcrystalline particles resulting from a poor dissolution of saquinavir or one of its pharmaceutical acceptable salts.  
15 The presence of microcrystalline forms in the super-concentrate mixtures like in the case of the presence invention, triggers the crystallization process of the active ingredient and this crystallization lower considerably the absorption of the active ingredient by the  
20 organism implicating directly in the bioavailability of the effective therapeutic dosage in the treatment of a viral infection. In the present invention the alcohol, preferably ethanol or propylene glycol or mixtures between them, will be used preferentially in a concentration ranging from 2% to  
25 20% in weight of the final composition.

The composition of the present invention has its formula improved by the presence of a non-ionic surfactant selected among the polyethoxylated ethers derivatives from castor oil, preferably the polyethoxylated castor oil 35  
30 (Chremophor EL), polyethoxylated hydrogenated castor oil (Cremophor RH 40), this last one presenting the advantage of being practically insipid when used in oral formulations. Other adequate surfactants that may be used in the present

invention are the polyoxyethylene sorbitan esters, compounds known as polysorbates. Among polysorbates used in the present invention, there are the liquid polysorbates like polysorbate 20, 40, 60 and 80. In the present invention  
5 surfactants are used in a concentration ranging from 0.1% to 30% in weight of the final composition.

In order to increase the stability of the composition of the present invention, substances known as antioxidants can be added to avoid its decomposition or accelerate  
10 degradation. Among adequate antioxidants useful to be used in the present invention are the alpha-tocopherol and the butylated hydroxytoluene (BHT). The use of alpha-tocopherol is advantageous in formulas of fatty acids because it exerts an adequate antioxidant activity to avoid the oxidation. In  
15 the composition of the present invention the antioxidant is used in a concentration ranging from 0.001% to 2.0% in weight of the final composition.

The pharmaceutical composition described in the present invention comprises a stable concentrate microemulsion  
20 wherein the active ingredient is completely dissolved. This concentrate microemulsion consists of a clear, transparent solution in the form of viscous oil.

The main characteristic of the composition of the present invention is its bioavailability profile  
25 surprisingly increased when compared to the existing marketed composition.

As disclosed before, saquinavir is a drug that its "in vivo" activity is very low due the poor bioavailability of the pharmaceutical compositions developed until now.  
30 Compared to other protease inhibitors, saquinavir is the substance that presents the lower bioavailability profile, being only just a small amount of the drug absorbed in the gastrointestinal tract and distributed to the patient

tissues. Due its behavior, today it is necessary the ingestion of several capsules from the medicine available in the market to reach the appropriate circulating therapeutic dose.

5       The composition of the present invention has a bioavailability profile several times higher than all pharmaceutical formulations developed until the present moment, allowing a marked reduction in the amount of capsules necessary to be ingested everyday and/or the  
10       reduction of the capsule volume in order to facilitate its ingestion.

      Considering the issue adhesion or adherence to the amount necessary of this medicine, the pharmaceutical composition of the present invention presents a  
15       bioavailability profile that contributes positively in the acceptance of the therapy by the patient. As disclosed before, a patient in treatment with saquinavir need to take up to 18 capsules of this medicine each day. The composition of the present invention allows the use of only three to  
20       five capsules of this medicine each day to reach the plasmatic levels suitable for the therapy, incisively contributing with the adherence of the patient to the amount of the drug prescribed and eliminating de discomfort of taking an excessive amount of medicines.

25       As innovator features of the this composition, special attention must be given to the bioavailability of saquinavir, which is at least five times higher than the composition now available in the market. Other innovative characteristic corresponds to the possibility of this  
30       composition being formulated comprising elevate quantities of saquinavir, without occurring its later precipitation or crystallization. Because of this aspect, the composition of the present invention can be formulated using saquinavir in

an amount ranging from 10% to 80% in weight of the final composition. Preferably the concentration range in the present invention will be from 15% to 70% in weight of the final composition, which corresponds to a concentration  
5 ranging from 150mg to 700mg of saquinavir per gram of the final composition. It corresponds to a dose 350% higher than the marketed composition, when considering its higher concentration.

By all these characteristics, the administration regime  
10 of this medicine can be improved and simplified. The quantity of capsules to be ingested can be lowered and/or the capsules can be miniaturized in order to have a more appropriate size to the ingestion.

Considering that the main factor for the non-adherence  
15 or partial adherence from patients to the treatment with protease inhibitors, specially saquinavir, is the quantity of capsules to be ingested daily and their uncomfortable size for the ingestion, the pharmaceutical composition of the present invention consists in an alternative extremely  
20 favorable for the adherence of the patient to treatment. Through the improvement achieved, it is possible the administration of higher quantities of saquinavir per capsule reducing considerably the amount of capsules to be taken each time.

25 Other alternative to increase the adhesion of patient to treatment is the possibility to promote the manufacture of a capsule with considerably reduced size, allowing its easy administration to patients with problems in ingesting the voluminous capsules now available in the market, even  
30 allowing the treatment of children that are not treated because they are not able to ingest the already available medicine.

Another objective of the present invention is the process for preparing pharmaceutical compositions with increased bioavailability, consisting in a concentrate microemulsion of saquinavir or its pharmaceutical acceptable salts.

Due the low solubility of saquinavir and/or its pharmaceutical acceptable salts, the preparing of the pharmaceutical composition can not be executed using a direct dissolution technique, or it means, it is not possible to obtain this pharmaceutical composition in the indicated concentrations by a procedure consisting basically in the dissolution of saquinavir or one of its salts in any of its ingredients or their indicated combinations, even when the active ingredient is micronized or submitted to elevate temperatures for prolonged periods of time in order to proportionate its dissolution.

The process for preparing pharmaceutical compositions constituted by a concentrate microemulsion of N-tert-butyldecahydro-2-[2(R)hydroxy - 4-phenyl-3-(S)-[[N- (2-quinolylcarbonyl) -L- asparaginy]amine]butyl]-(4aS,8aS)-isoquinolone-3(S)-carboxamide (saquinavir), or its pharmaceutical acceptable salts, comprises the following steps:

- a) Dissolving completely of N-tert-butyldecahydro-2-[2(R)hydroxy - 4-phenyl-3-(S)-[[N- (2-quinolylcarbonyl) -L- asparaginy]amine]butyl]-(4aS,8aS)-isoquinolone-3(S)-carboxamide , or its pharmaceutical acceptable salt, in a sufficient amount of the alcohol of C<sub>2-4</sub> under controlled temperature;
- b) Eliminating particles by filtration;



- 5           c) Adding the fatty acid, the antioxidant and the surfactant in an appropriate amount used in the composition;
- d) Evaporating the alcohol at a maximum temperature of 50°C under reduced pressure;
- e) Optionally, adding the surfactant from step (c) after the evaporation of the alcohol from step (d);
- 10          f) Adding the alcohol C<sub>2-4</sub> under stirring and in an enough amount to complete the adequate weight of the final composition.

Initially saquinavir, or its pharmaceutical acceptable salt, is completely dissolved in sufficient amount of alcohol necessary to obtain a completely clear solution, In order to avoid any degradation of the active ingredient, 15 this dissolution is performed in a temperature ranging from 20°C to 50°C, under stirring. To guarantee the absence of solid particles that can trigger the later precipitation process, this alcoholic solution is filtered using usual filtration techniques and to the clear resulting solution 20 are added the fatty acid and the antioxidant, being the resulting solution concentrated at reduced pressure employing a maximum temperature of about 50°C. To the resulting concentrate is added the surfactant and the mixture is stirred until it turns into a clear oily liquid.

25          Optionally the surfactant may be added with the fatty acid and the antioxidant together. The final correction of the composition is done by adding the alcohol until the composition reaches the weight concentration desired of saquinavir or its pharmaceutical acceptable salt.

30          The alcohol used in the initial dissolution of saquinavir is preferably the ethanol, because its evaporation can be easily done under low temperatures at

reduced pressures employing usual industrial techniques. After the addition of the other components, the fatty acid, the antioxidant and the surfactant, the alcohol used to complete the final weight of the composition is preferably  
5 ethanol, or propylene glycol or mixtures between them.

In accordance to the process, the resulting alcoholic solution from saquinavir dissolution presents a saquinavir concentration ranging from 0.01% to 90% in weight of the final solution. The alcohol preferably employed is the  
10 ethanol, but other alcohols of C<sub>2-4</sub> may be used, namely methanol, isopropanol, propanol and butanols. The alcohol used for preparing the saquinavir solution is preferably used in a concentration ranging from 10% to 99.99% in weight of the final solution. The temperature used for dissolving  
15 saquinavir in the alcohol ranges from 20°C to 50°C, temperatures mild enough to avoid saquinavir degradation. More elevate temperatures may be used, taking the appropriate care to monitor saquinavir stability in the solution.

20 After dissolving saquinavir in the alcohol, the resulting solution is filtered using usual industrial filtration techniques. Filtration through microporous membranes is particularly interesting, eliminating eventual microparticles that could remain in the solution.

25 Excipients comprising the final composition are add after the filtration, being them the fatty acid of C<sub>12-18</sub>, the antioxidant and the surfactant that can be add optionally in this step or after the next step consisting in the alcohol evaporation.

30 Alcohol elimination by evaporation is preferably performed under reduced pressure, so controlling the medium temperature avoiding degradation of the active ingredient and/or the excipients added. Alcohol elimination is

performed until the composition reaches its appropriate weight or it can be removed until the composition reaches a weight lower than desired, the final composition weight being reached by adding one or more alcohols of  $C_{2-4}$ .

5       The product resulting from the process disclosed above presents as its final composition saquinavir in a concentration ranging from 10% to 80%, preferably in a concentration ranging from 15% to 75% in weight of the final composition. The fatty acid of chain  $C_{12-18}$  is present in a  
10       concentration ranging from 20% to 80% in weight of the final composition, preferably in a concentration ranging from 20% to 70% in weight of the final composition. It presents one or more alcohols of  $C_{2-4}$  in a concentration ranging from 2.0% to 20% in weight of the final composition. It presents the  
15       non-ionic surfactant selected among polyethoxylated castor oil derivatives, preferably polyethoxylated castor oil 35 (Cremophor 35) or polyethoxylated hydrogenated castor oil 40 (Cremophor RH 40) or among polyoxyethylene sorbitan esters (polysorbates), preferably liquid polysorbates at ambient  
20       temperatures like polysorbates 20, 40, 60 or 80 in a concentration ranging from 0.1% to 30% in weight of the final composition. Finalizing, the product resulting from the described process presents as antioxidant the alpha-tocopherol or butylated hydroxytoluene in a concentration  
25       ranging from 0.001% to 2.0% in weight of the final composition.

By this procedure it is possible to obtain a pharmaceutical composition of a stable concentrate microemulsion of saquinavir, or one of its acceptable salt,  
30       wherein the active ingredient is completely dissolved, without the presence of saquinavir microcrystalline forms, that are able to trigger the crystallization of the active

ingredient on standing, which would interfere in the absorption and the bioavailability of the medicine.

By using this process there is no need to use saquinavir or one of its pharmaceutical acceptable salts in  
5 a special crystalline form as, for example, in an amorphous or micronized form in order to allow its complete dissolution in the selected excipients. Any crystalline form can be used without interfering on its stability over crystallization from the final composition.

10 Additionally, by this technical advance, it is possible to prepare highly concentrate compositions, impossible to prepare by direct dissolution procedures using the excipients described.

The concentrate microemulsion resulting from this  
15 process presents high stability over precipitation and/or crystallization and over changes in its physical state. Even when the composition is submitted to low temperatures (4°C to 8°C) for long periods of time, there is no sign of gel formation or the appearance of crystalline or  
20 microscristalline formation, which would trigger the precipitation or crystallization of the active ingredient, and such event would damage the absorption and bioavailability of the protease inhibitor, which would not be soluble in the composition.

25 The stability of the present invention composition over crystallization of saquinavir grants its delivery or liberation in the absorption place in a soluble form, adequate to its prompt absorption by the organism.

Stability monitoring of the concentrate microemulsion  
30 composition shows a reliable result on considering the ability of keeping the active ingredient in a soluble state suitable for the prompt absorption of the drug by the

organism. During the studies it was not observed the formation or transformation of the solution into a jelly composition, as it happens with the marketed capsules available today, demonstrating that the pharmaceutical  
5 composition of the present invention presents a physical form stable and adequate to the liberation and dissolution in a constant rate.

The composition of the present invention comprising a concentrate microemulsion can be employed in a final  
10 pharmaceutical presentation consisting by soft or hard gelatin capsules.

In a preferential realization of the present invention, a pharmaceutical composition consisting of a concentrate microemulsion disclosed above is encapsulated in soft  
15 gelatin capsules that present uniform liberation properties of its contents inside the gastrointestinal tract, as well presenting a better receptivity from the patient because of its elastic properties that allows an easier ingestion.

The technique for preparing the soft gelatin capsule is very well known basically consisting on using gelatin,  
20 plasticizing agent and water in definite proportions. Additionally the capsule material may contain additives like inks, pigments and flavors, among others. The manufacture of soft gelatin capsules comprises several techniques, like,  
25 for example, a process with or without sewing, rotatory, using specific machinery, among others. Soft gelatin capsules used in this invention as a film for covering the concentrate pharmaceutical formulation consist in  
30 pharmaceutical gelatin, glycerol, propylparabene, titanium dioxide and water, and they were prepared by conventional technique.

As a general rule the composition of the present invention can be submitted to all of the existing processes

of producing soft gelatin capsules since they do not considerably interfere with the composition, it means, that such process of producing does not considerably change the ratio among its ingredients by evaporation because heat exposition, drying processes or any other kind of processing.

The following examples are illustrative, but not exhaustive about the possibilities of the composition of the present invention and its process for preparing, as well the tests that demonstrate its stability and the maintaining of its properties of a soluble concentrate microemulsion of saquinavir on standing, and its improved bioavailability profile.

**Example 1a: Preparation of a 20% saquinavir composition**

In a 2L reactor add 200g (20.00%) of saquinavir and 1,000mL of absolute ethanol. Keep the system under stirring at a temperature up to 50°C until all solids dissolution. This solution is filtered to eliminate solid particles and to the filtrate are add oleic acid (526.2g - 52.2%) and tocopherol (7.44g - 0.744%). This mixture is stirred for 5 minutes and then is vacuum concentrated at a temperature up to 50°C. To the resulting concentrate, polyethoxylated castor oil 35 is added (141.8 -14.18%) and the final weight of the solution is brought to 1,000g with absolute ethanol, if necessary.

By this way prepared, the final composition presents a concentration of 200mg of saquinavir for each 1g of the composition. It is a clear and yellow oily liquid. When stored under refrigeration (4°C to 8°C) for 120 days it does not present crystals or any dispersed solid formations, maintaining its clear aspect.

**Example 1b: Preparation of a 20% saquinavir composition with propylene glycol**

For preparing a concentrate composition of saquinavir comprising propylene glycol as the alcoholic excipient, it is used the procedure described in Example 1a, but replacing the alcohol used to complete the final weight of the resulting composition by propylene glycol.

**Example 2a: Preparation of a 40% saquinavir composition**

In a 5L reactor add 400g (40%) of saquinavir and 2,000mL of absolute ethanol. Keep the system under stirring in a temperature up to 50°C until all solids dissolution. This solution is filtered to eliminate solid particles and to the filtrate are added the oleic acid (370g - 37%), the tocopherol (7.44g - 0.744%) and the polyethoxylated castor oil 35 (100g - 10%). This mixture is stirred for 5 minutes and then is concentrated under vacuum at a temperature up to 50°C. The final weight of the solution is corrected to 1,000g with absolute ethanol if necessary.

By this way prepared, the final composition presents a concentration of 400mg of saquinavir for each 1g of the composition. It is a clear and yellow oily liquid. When stored under refrigeration (4°C to 8°C) for 120 days it does not present crystals or any dispersed solid formations, maintaining its clear aspect.

**Example 2b: Preparation of a 40% saquinavir composition with propylene glycol**

For preparing a concentrate composition of saquinavir comprising propylene glycol as the alcohol as excipient, it is used the procedure described in Example 2a, but replacing the alcohol used to complete the final weight of the resulting composition by propylene glycol.

**Example 3a: Preparation of a 60% saquinavir composition**

In a 5L reactor add 600g (60%) of saquinavir and 3,000mL of absolute ethanol. Keep the system under stirring in a temperature ranging from 20°C to 50°C untill all solids  
5 dissolution. This solution is filtered to eliminate solid particles and to the filtrate are added the oleic acid (200g - 20%) and the tocopherol (7.44g - 0.744%). This mixture is stirred for 5 minutes and then is concentrated under vacuum at a temperature up to 50°C. To this concentrate is added  
10 the polyethoxylated castor oil 35 (150g - 15%) and the final weight of the solution is corrected to 1,000g with absolute ethanol, if necessary.

By this way prepared, the final composition presents a concentration of 600mg of saquinavir for each 1g of the  
15 composition. It is a clear and yellow oily liquid. When stored under refrigeration (4°C to 8°C) for 120 days it does not present crystals or any dispersed solid formations, maintaining its clear aspect.

**Example 3b: Preparation of a 60% saquinavir composition with propylene glycol**  
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For preparing a concentrate composition of saquinavir comprising propylene glycol as the alcohol as excipient, it is used the procedure described in Example 3a, but replacing the alcohol used to complete the final weight of the  
25 resulting composition by propylene glycol.

By using the process described in the above examples there were prepares the compositions disclosed in the Table 1 below.



**Table 1: Examples of saquinavir concentrate compositions prepared accordingly with the present invention**

<i>Composition</i>		<i>Used quantities</i>
C1	Saquinavir base	200g
	Oleic acid	526.2g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	141.8g
	Absolute Ethanol	s.q.t. 1,000g
C2	Saquinavir base	200g
	Oleic acid	450g
	Tocopherol	5.0g
	Polyethoxylated castor oil 35	225g
	Absolute Ethanol	s.q.t. 1,000g
C3	Saquinavir mesylate	228.65g
	Oleic acid	505g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	134g
	Absolute Ethanol	s.q.t. 1.000g
C4	Saquinavir base	600g
	Oleic acid	555g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	150g
	Absolute Ethanol	s.q.t.* 1,500g
C5	Saquinavir base	600g
	Oleic acid	200g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	150g
	Absolute Ethanol	s.q.t.* 1,000g
C6	Saquinavir Mesylate	685.96g
	Oleic acid	500g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	120g
	Absolute Ethanol	s.q.t. 1,500g
C7	Saquinavir base	600g
	Oleic acid	230g
	Tocopherol	3.7g
	Polyethoxylated castor oil 35	125g
	Absolute Ethanol	s.q.t. 1,000g
C8	Saquinavir base	600g
	Oleic acid	555g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	150g
	Propyleneglycol	s.q.t. 1,500g
C9	Saquinavir base	600g
	Oleic acid	555g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	200g
	Absolute Ethanol	s.q.t. 1,500g
C10	Saquinavir Masylate	685.96g
	Oleic acid	430g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	200g
	Absolute Ethanol	s.q.t. 1,500g
C11	Saquinavir base	600g
	Oleic acid	200g
	Tocopherol	7.44g
	Polyethoxylated castor oil 35	68g
	Absolute Ethanol	s.q.t. 1,000g

C12	Saquinavir base Oleic acid Tocopherol Polyethoxylated castor oil 35 Propylenoglycol/ethanol (1:1 volume)	600g 555g 7.44g 150g s.q.t.* 1,500g
C13	Saquinavir base Oleic acid Tocopherol Polyethoxylated castor oil 35 Propylenoglycol/ethanol (1:1 volume)	600g 200g 7.44g 150g s.q.t.* 1,000g
C14	Saquinavir base Oleic acid Tocopherol Polyethoxylated castor oil 40 Absolute ethanol	600g 750g 7.44g 20g s.q.t.* 1,500g
C15	Saquinavir base Oleic acid Tocopherol Polyssorbate 40 Absolute ethanol	600g 555g 7.44g 150g s.q.t.* 1,500g

s.q.t. - Suficient Quantity To complete the desired weight.

#### BIOAVAILABILITY COMPARATIVE STUDY

The bioavailability profile of composition C1 from the present invention was evaluated in human volunteers in comparison with FORTOVASE™ composition.

The study consisted in the administration of a single dose of 200mg of saquinavir from the composition C1 of the present invention and from the FORTOVASE™ composition. Individuals were submitted to a two confinement periods of 25 hours, with a rest period of one week between them, and all individuals received both compositions in a random way according to the design of the study.

Twelve healthy volunteers from both sexes were used in the study, each one of them receiving a 200mg FORTOVASE™ composition or a 200mg of composition C1. Volunteers received the medication in a fasting state followed by 200mL of water. Fasting was established by a two hours period after the administration of the dosae, and after that it was served a standard breakfast free from xanthine. Five hours after the administration of the drugs another meal was offered, 8 hours after administration was offered a lunch

and another meal at 11 hours after administration, all the meals free from xanthine. Liquid refreshments were free allowed, but xanthine-containing liquids were prohibited.

Alcohol use was forbidden from the last 48 hours before the beginning of the study. Use of cigarettes, cigars and similar were not allowed during the study.

Saquinavir plasma concentration was determined by high performance liquid chromatography with mass spectrometry (LC-MS-MS). Blood samples for the assay of the drug in the plasma were collected at 0.33; 0.67; 1; 1.33; 1.67; 2; 2.33; 2.67; 3; 3.5; 4; 4.5; 5; 5.5; 6; 7; 8; 10 and 12 hours after the administration of the medicine.

The maximum concentration ( $C_{max}$ ) observed in the plasma and the time to achieve the maximum concentration were determined for each composition. Areas under the time-concentration curve were calculated by using the linear regression log trapezoidal, except for  $AUC_{0-12h}$ , where it was used the trapezoidal method.

Table 2 below summarizes the pharmacokinetics parameters from the inventive composition C1 against the reference medicine FORTOVASE<sup>™</sup>.

**Table 2: Results from the comparative bioavailability study.**

	<b>FORTOVASE<sup>®</sup></b>	<b>Inventive composition</b>
$C_{max}$	11.26	64.48
$T_{max}$	0.67	0.67
$AUC_{0-12}$	13.20	69.58

Data from Table 2 reveal that the inventive composition C1 presents a  $C_{max}$  almost six times higher and an  $AUC_{0-12h}$  five times higher than the same parameters found for the

reference composition (FORTOVASE<sup>™</sup>). These data are surprisingly, indicating an increased bioavailability when comparing the inventive composition against the reference composition.

- 5        Figure 1 presents the comparative plot of the mean concentration of saquinavir from the present invention composition C1, and the saquinavir from reference FORTOVASE<sup>™</sup> composition over time.